

Listing of Claims:

Claim 1 (cancelled)

Claim 2 (withdrawn): A method of producing a hybrid seed with restored male fertility from plants selected from those species of pollen producing plants which are capable of being genetically transformed comprising the steps of:

- (a) producing a plant with a male sterile trait by a method comprising the steps of:
 - (1) transforming a plant cell of said plant with a sense gene which confers on said plant resistance to a chemical agent or a naturally occurring or artificially induced physiological or chemical stress;
 - (2) regenerating from said transformed plant cell a genetically transformed plant which is resistant to the same stress;
 - (3) inserting into the genome of a plant cell of the stress resistant plant a recombinant DNA molecule comprising:
 - (i) a DNA sequence that codes for RNA that is complementary to the RNA sequence encoded by the said sense gene;
 - (ii) a pollen specific promoter which functions in said plant cell to cause transcription of said DNA sequence into RNA; and
 - (iii) a terminator sequence which defines a termination signal during transcription of said DNA sequence;
 - (4) obtaining a plant cell of said stress resistant plant which has been transformed with the gene described in step (c) above; and
 - (5) regenerating from said transformed plant cell a plant which has been genetically transformed with the genes described in step (a) and step (c) above and can be rendered male sterile by said chemical agent or stress; and
- (b) increasing the number of genetically transformed male sterile plants by
 - (i) clonal propagation of said genetically transformed male sterile plant obtained in step (a) using tissue explants thereof, or other *in vitro* propagation techniques; or

- (ii) crossing said genetically transformed male sterile plant with a suitable male fertile plant;
 - (iii) using said chemical agent or physiological stress to eliminate plants which are not genetically transformed with the DNA sequence amongst plants grown from seed produced by such cross; and
 - (iv) repeating such cross over several generations with plants obtained in step (b)(iii) above in the presence of said chemical agent or physiological stress to increase the numbers of male sterile plants;
- (c) producing a male fertile restorer plant by:
- (i) inserting into the genome of a plant cell of a suitable male parent plant that is capable of regeneration into a differentiated whole plant a gene that confers resistance to a chemical agent or a naturally occurring or artificially induced physiological stress, linked to a recombinant DNA sequence comprising:
 - A. a recombinant DNA molecule that comprises a modified form of said sense gene that does not contain the regions complementary to said antisense gene;
 - B. a promoter that functions in said plant to cause transcription of said modified DNA sequence at a time which restores the function of said sense gene, preferably at or about the time of the action of the anti-sense gene; and
 - C. a terminator sequence which defines a termination signal during transcription of said DNA sequence;
- (d) increasing the number of genetically transformed male fertile restorer plants in the fashion described in (b) above or by selecting a plant homozygous for said restorer trait and increasing said plant by selfing in isolation; and
- (e) effecting a hybrid cross by pollinating said male sterile plants with pollen from said male fertile restorer plants.

Claims 3 - 7 (cancelled)

Claim 8 (withdrawn): A method of producing seed of a male sterile plant comprising:

- (a) producing a male sterile plant line comprising
 - (i) introducing into the genome of one or more plant cells of a pollen-producing plant a first recombinant DNA molecule comprising:
 - (1) one or more DNA sequences which may be the same or different, which encode a gene product which when produced in a cell of a plant which is essential to pollen formation and/or function is directly or indirectly capable of substantially interfering with the function and/or development of said cell; and
 - (2) one or more promoters which may be the same or different, said promoters being capable of regulating the expression of said DNA sequences; and

wherein the DNA sequences and promoters are selected such that the gene product selectively interferes with the function and/or development of a cell of a plant that is essential to pollen formation and/or function and whereby a plant regenerated from a cell of a plant having said recombinant DNA molecule integrated into its nuclear genome is substantially male-sterile or carries the male sterile trait

said first recombinant DNA molecule comprises a pollen specific promoter and a selection marker gene which encodes a selection gene product which permits the selection of a plant having said first recombinant DNA molecule integrated in its genome;

- (ii) selecting a plant cell into which the first recombinant DNA molecule is stably integrated;
- (iii) regenerating from the selected plant cell a plant which carries the male sterile trait;
- (iv) increasing the number of plants which carry the male sterile trait to produce a plant line having plants carrying the male sterile trait; and
- (v) exposing said plant line to the non-toxic substance to render plants of said plant line male sterile;

(b) cross pollinating plants of the male sterile plant line obtained in (a) with plants of a second plant line having a genome which stably incorporates a second recombinant DNA molecule comprising a second DNA sequence encoding a second DNA gene product which is capable of converting a substance which is endogenous to cells of plants of said second plant line, to said non-toxic substance; a second promoter capable of regulating the expression of said second DNA sequence; said first and second recombinant DNA molecules are incorporated into homologous chromosome pairs, wherein plants of said second plant line are not capable of rendering the non-toxic substance cytotoxic to cells of plants of said second line which are essential to pollen formation and/or function; and

(c) harvesting seed of plants of said male sterile plant line.

Claims 9 – 12 (cancelled)

Claim 13 (previously presented): A method to produce hybrid seed with restored male fertility comprising the steps of:

(a) inserting into the genome of a plant cell of a pollen producing plant a gene which confers on said plant resistance to an herbicide or antibiotic, and linked to said gene a recombinant DNA molecule comprising:

- (i) a DNA sequence which codes for a cytotoxic molecule;
- (ii) a promoter capable of regulating the transcription of said DNA sequence in cells or tissues critical to pollen formation or function; and
- (iii) a terminator sequence which defines a terminal signal during transcription of such DNA sequence;

(b) obtaining a transformed plant cell;

(c) regenerating from said plant cell a genetically transformed plant which is male sterile;

(d) increasing the number of genetically transformed plants by:

- (i) crossing the genetically transformed plant described in step (c) above with a suitable male fertile plant;

- (ii) using an herbicide or antibiotic to eliminate plants which do not contain the genes described in step (a) among plants grown from seed produced by such cross; and
 - (iii) repeating such a cross over several generations with the plants obtained as in step (d)(ii) above in the presence of said herbicide or antibiotic to increase the numbers of male sterile plants;
- (e) inserting into a plant cell of a suitable male fertile plant selected from the same species a gene which confers on said plant resistance to an herbicide or antibiotic and linked to said gene a recombinant DNA molecule comprising:
- (i) a DNA sequence which codes for RNA that is complementary to the RNA sequence coding for said cytotoxic molecule; and
 - (ii) a promoter which causes transcription of the DNA sequence defined in step (e)(i) above at or about the time of transcription of the DNA sequence defined in step (a)(i);
- (f) obtaining a transformed plant cell from step (d);
- (g) regenerating from said transformed plant cell described in step (d) above a genetically transformed male fertile plant; and
- (h) producing a restorer line by:
- (i) selfing the genetically transformed plant described in (g) and selecting from that selfing progeny, a plant homozygous for the male restorer trait;
 - (ii) permitting self-fertilization of said plant homozygous for the male restorer trait;
 - (iii) growing seed of said plant, over a number of generations to increase the number of genetically transformed plants; and
 - (iv) effecting a hybrid cross by pollinating said male sterile plants with pollen from said genetically transformed male fertile plants.

14. (previously presented): The method of claim 13, wherein said recombinant DNA molecule defined in step (e) further comprises a terminator sequence which defines a termination signal during transcription of the DNA sequence described in step (e)(i).

15. (previously presented): A method of producing hybrid seed with restored male fertility comprising the steps of:

- (a) (i) inserting into the genome of a plant cell of a plant that is capable of regeneration into a differentiated whole plant, a sense gene that confers resistance to an herbicide or antibiotic and linked to this a recombinant DNA molecule comprising:
 - A. a DNA sequence that when transcribed and translated codes for a cytotoxic molecule or a molecule which breaks down a substance into a cytotoxic molecule;
 - B. a promoter capable of regulating the transcription of said DNA sequence into RNA at or about the time of the transcription of the sense gene in cells or tissues critical to pollen formation or function; and
 - C. a terminator sequence which defines a termination signal during transcription of said DNA sequence;
 - (ii) obtaining a transformed plant cell of said plant; and
 - (iii) regenerating from said plant cell a plant which is genetically transformed with said DNA sequences described in (a)(i) above and is male sterile; and
- (b) increasing the number of genetically transformed male sterile plants by:
- (i) clonal propagation of said genetically transformed male sterile plant described in step (a) using tissue explants thereof, or other *in vitro* propagation techniques; or
 - (ii) A. crossing the genetically transformed male sterile plant described in (a) with a isogenic male fertile plant;

- B. using an herbicide or antibiotic to eliminate plants which do not contain the DNA sequence defined in (a) (i) amongst plants grown from seed produced by such cross; and
 - C. repeating such cross over several generations with plants obtained in step (a)(iii) above in the presence of said herbicide or antibiotic to increase the numbers of male sterile plants;
- (c) producing a male fertile restorer plant by:
- (i) inserting into the genome of a plant cell of a suitable male parent plant that is capable of regeneration into a differentiated whole plant a gene that confers resistance to an herbicide or antibiotic, linked to a recombinant DNA sequence comprising:
 - A. a gene that codes for a molecule that negates the disruption caused to cells or tissues critical to pollen formation or function in said genetically transformed female parent plant;
 - B. a promoter that functions in said cells or tissues critical to pollen formation or function to cause transcription of said gene into RNA at or about the time that the sense gene described in (a)(i) is active;
- (d) increasing the number of genetically transformed male fertile restorer plants by:
- (i) selfing the genetically transformed plant carrying the restorer trait described in (c), and selecting a plant homozygous for the restorer trait and increasing said plant by selfing in isolation; or
 - (ii) conducting anther or isolated microspore culture of the genetically transformed plant carrying the restorer trait described in (c) and selecting a plant homozygous for the restorer trait and increasing said plant by selfing in isolation.

16. (previously presented): The method of claim 15, wherein said recombinant DNA molecule defined in step (c)(i) further comprises a terminator sequence which defines a termination signal during transcription of the DNA sequence described in step (c)(i)A.